

What Is Claimed Is:

1. A serum-free, eukaryotic cell culture medium supplement comprising one or more ingredients selected from the group consisting of albumins or albumin substitutes, one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein a basal cell culture medium supplemented with said supplement is capable of supporting the growth of embryonic stem cells in serum-free culture.

2. A serum-free, eukaryotic cell culture medium supplement comprising an albumin or an albumin substitute and one or more ingredients selected from group consisting of one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein a basal cell culture medium supplemented with said supplement is capable of supporting the growth of embryonic stem cells in serum-free culture.

3. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said antioxidant is selected from the group consisting of reduced glutathione and ascorbic acid an ascorbic acid-2-phosphate.

4. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said collagen precursor is selected from the group consisting of L-proline and multimers or derivatives thereof, L-hydroxyproline multimers or derivatives thereof, and ascorbic acid and multimers thereof.

5. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said transferrin substitute is an iron chelate selected from the group consisting of a ferric citrate chelate and a ferrous sulfate chelate.

5 6. The serum-free, eukaryotic cell culture medium supplement according to claim 5, wherein said transferrin substitute is ferrous sulphate·7 water·EDTA.

7. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said insulin substitute is selected from the group consisting of zinc chloride, zinc bromide, and zinc sulfate·7 water.

10 8. The serum-free, eukaryotic cell culture medium supplement according to claim 7, wherein said insulin substitute is zinc sulfate·7 water.

15 9. The serum-free, eukaryotic cell culture medium supplement formulation according to claim 1, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of glycine, L-alanine, L-asparagine, L-cysteine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-glutamine, L-arginine, L-methionine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, and L-valine, and derivatives thereof.

20 10. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said albumin substitute is selected from the group consisting of bovine pituitary extract, plant hydrolysate, fetal calf albumin (fetuin), egg albumin, human serum albumin (HSA), chick extract, bovine embryo extract, AlbuMAX® I, and AlbuMAX® II.

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11. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said albumin substitute is AlbuMAX® I.

12. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said trace element ingredient comprises one or more trace element moieties selected from the group consisting of Ag^+ , Al^{3+} , Ba^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Ge^{4+} , Se^{4+} , Br^- , I^- , Mn^{2+} , F^- , Si^{4+} , V^{5+} , Mo^{6+} , Ni^{2+} , Rb^+ , Sn^{2+} and Zr^{4+} .

13. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said supplement is concentrated.

14. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said supplement is concentrated from about 2-fold to about 10-fold.

15. The serum-free, eukaryotic cell culture medium supplement according to claim 1, wherein said supplement is added to a basal medium to a final concentration of about 0.5% to about 90%.

16. The serum-free, eukaryotic cell culture medium supplement according to claim 15, wherein said supplement is added to a basal medium to a final concentration of about 5% to about 50%.

17. The serum-free, eukaryotic cell culture medium supplement according to claim 16, wherein said supplement is added to a basal medium to a final concentration of about 5% to about 30%.

18. The serum-free, eukaryotic cell culture medium supplement according to claim 17, wherein said supplement is added to a basal medium to a final concentration of about 5% to about 20%.

19. The serum-free, eukaryotic cell culture medium supplement according to claim 18, wherein said supplement is added to a basal medium to a final concentration of about 15%.

20. A serum-free, eukaryotic cell culture medium supplement obtained by combining an albumin or an albumin substitute and one or more ingredients selected from group consisting of one or more amino acids, one or more vitamins, one or more transferrins or transferrin substitutes, one or more antioxidants, one or more insulins or insulin substitutes, one or more collagen precursors, and one or more trace elements,

wherein a basal cell culture medium supplemented with the supplement is capable of supporting the growth of embryonic stem cells in serum-free culture.

21. A serum-free, eukaryotic cell culture medium supplement comprising AlbuMAX® I, glycine, L-histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, sodium selenite, Ag^+ , Al^{3+} , Ba^{2+} , Cd^{2+} , Co^{2+} , Cr^{3+} , Ge^{4+} , Se^{4+} , Br^- , I^- , Mn^{2+} , F^- , Si^{4+} , V^{5+} , Mo^{6+} , Ni^{2+} , Rb^+ , Sn^{2+} and Zr^{4+} ,

wherein a basal cell culture medium supplemented with said supplement is capable of supporting the growth of embryonic stem cells in serum-free culture.

22. A serum-free, eukaryotic cell culture medium supplement obtained by combining water, AlbuMAX® I, glycine, L-histidine·HCl·water, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-

ascorbic acid-2-phosphate, iron saturated transferrin, insulin, sodium selenite, a Ag^+ salt, an Al^{3+} salt, a Ba^{2+} salt, a Cd^{2+} salt, a Co^{2+} salt, a Cr^{3+} salt, a Ge^{4+} salt, a Se^{4+} salt, a Br^- salt, an I^- salt, a Mn^{2+} salt, a F^- salt, a Si^{4+} salt, a V^{5+} salt, a Mo^{6+} salt, a Ni^{2+} salt, a Rb^+ salt, a Sn^{2+} salt, and a Zr^{4+} salt,

wherein each ingredient is present in an amount which, when added to a basal medium, supports the growth of embryonic stem cells in serum-free culture.

23. The serum-free, eukaryotic cell culture medium supplement according to claim 22, wherein said Ag^+ salt is AgNO_3 , said Al^{3+} salt is $\text{AlCl}_3 \cdot 6$ water, said Ba^{2+} salt is $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2$, said Cd^{2+} salt is $\text{CdSO}_4 \cdot 8$ water, said Co^{2+} salt is $\text{CoCl}_2 \cdot 6$ water, said Cr^{3+} salt is $\text{Cr}_2(\text{SO}_4)_3 \cdot 1$ water, said Ge^{4+} salt is GeO_2 , said Se^{4+} salt is both Na_2SeO_3 and H_2SeO_3 , said Br^- salt is KBr , said I^- salt is KI , said Mn^{2+} salt is $\text{MnCl}_2 \cdot 4$ water, said F^- salt is NaF , said Si^{4+} salt is $\text{Na}_2\text{SiO}_3 \cdot 9$ water, said V^{5+} salt is NaVO_3 , said Mo^{6+} salt is $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4$ water, said Ni^{2+} salt is $\text{NiSO}_4 \cdot 6$ water, said Rb^+ salt is RbCl , said Sn^{2+} salt is SnCl_2 , and said Zr^{4+} salt is $\text{ZrOCl}_2 \cdot 8$ water.

24. A method of making a serum-free, eukaryotic cell culture medium supplement, said method comprising admixing water, AlbuMAX® I, glycine, L-histidine, L-isoleucine, L-methionine, L-phenylalanine, L-proline, L-hydroxyproline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, thiamine, reduced glutathione, L-ascorbic acid-2-phosphate, iron saturated transferrin, insulin, sodium selenite, a Ag^+ salt, an Al^{3+} salt, a Ba^{2+} salt, a Cd^{2+} salt, a Co^{2+} salt, a Cr^{3+} salt, a Ge^{4+} salt, a Se^{4+} salt, a Br^- salt, an I^- salt, a Mn^{2+} salt, a F^- salt, a Si^{4+} salt, a V^{5+} salt, a Mo^{6+} salt, a Ni^{2+} salt, a Rb^+ salt, a Sn^{2+} salt, and a Zr^{4+} salt,

wherein each ingredient is present in an amount which, when added to a basal medium, supports the growth of embryonic stem cells in serum-free culture.

25. The method according to claim 23, wherein said Ag^+ salt is AgNO_3 , said Al^{3+} salt is $\text{AlCl}_3 \cdot 6$ water, said Ba^{2+} salt is $\text{Ba}(\text{C}_2\text{H}_3\text{O}_2)_2$, said Cd^{2+} salt is $\text{CdSO}_4 \cdot 8$ water, said Co^{2+} salt is $\text{CoCl}_2 \cdot 6$ water, said Cr^{3+} salt is $\text{Cr}_2(\text{SO}_4)_3 \cdot 1$ water, said Ge^{4+} salt is GeO_2 , said Se^{4+} salt is both Na_2SeO_3 and H_2SeO_3 , said Br^- salt is KBr , said I^- salt is KI , said Mn^{2+} salt is $\text{MnCl}_2 \cdot 4$ water, said F^- salt is NaF , said Si^{4+} salt is $\text{Na}_2\text{SiO}_3 \cdot 9$ water, said V^{5+} salt is NaVO_3 , said Mo salt is $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4$ water, said Ni^{2+} salt is $\text{NiSO}_4 \cdot 6$ water, said Rb^+ salt is RbCl , said Sn^{2+} salt is SnCl_2 , and said Zr^{4+} salt is $\text{ZrOCl}_2 \cdot 8$ water.

26. A serum-free eukaryotic cell culture medium comprising a basal cell culture medium supplemented with the serum-free cell culture supplement according to claim 1,

wherein said supplemented culture medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

27. The serum-free eukaryotic cell culture medium according to claim 26, wherein said medium is a 1X medium formulation.

28. The serum-free eukaryotic cell culture medium according to claim 26, wherein said medium is a concentrated medium formulation.

29. The serum-free, eukaryotic cell culture medium according to claim 26, wherein the final concentration of said supplement is about 0.5% to about 90%.

30. The serum-free, eukaryotic cell culture medium according to claim 29, wherein the final concentration of said supplement is about 5% to about 50%.

31. The serum-free, eukaryotic cell culture medium according to claim 30, wherein the final concentration of said supplement is about 5% to about 30%.

32. The serum-free, eukaryotic cell culture medium according to claim 31, wherein the final concentration of said supplement is about 5% to about 20%.

33. The serum-free, eukaryotic cell culture medium according to claim 30, wherein the final concentration of said supplement is about 15%.

5 34. A serum-free eukaryotic cell culture medium obtained by combining a basal cell culture medium with the serum-free supplement according to claim 1,

wherein said medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

10 35. A method of making a serum-free eukaryotic cell culture medium, said method comprising admixing a basal cell culture medium with the supplement according to claim 1,

wherein said medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

15 36. The method according to claim 35, wherein said medium is a 1X formulation.

37. The method according to claim 35, wherein said medium is a concentrated formulation.

20 38. The serum-free, eukaryotic cell culture medium according to the method of claim 35, wherein the final concentration of said supplement is about 0.5% to about 90%.

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39. The serum-free, eukaryotic cell culture medium according to the method of claim 38, wherein the final concentration of said supplement is about 5% to about 50%.

40. The serum-free, eukaryotic cell culture medium according to the method of claim 39, wherein the final concentration of said supplement is about 5% to about 30%.

41. The serum-free, eukaryotic cell culture medium according to the method of claim 40, wherein the final concentration of said supplement is about 5% to about 20%.

42. The serum-free, eukaryotic cell culture medium according to the method of claim 41, wherein the final concentration of said supplement is about 15%.

43. A composition comprising embryonic stem cells in a serum-free medium, wherein said serum-free medium is capable of supporting the growth of embryonic stem cells in serum-free culture.

44. The composition according to claim 43, wherein said medium is the medium according to claim 26 or 34.

45. The composition according to claim 44, wherein said composition is capable of being stored indefinitely at less than or equal to about -135°C .

46. The composition according to claim 45, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of human, monkey, ape, mouse, rat, hamster, rabbit, guinea pig, cow, swine, dog, horse, cat, goat, sheep, bird, reptile, fish, and amphibian.

47. The composition according to claim 46, wherein said embryonic stem cells are obtained from an animal selected from the group consisting of mouse, cow, goat, and sheep.

48. The composition according to claim 47, wherein said embryonic stem cells are obtained from mouse.

49. A product of manufacture comprising a container means containing embryonic stem cells and the supplement according to claim 1.

50. A product of manufacture comprising a container means containing embryonic stem cells in the medium according to claim 26 or 34.

51. A product of manufacture comprising one or more container means, wherein a first container means contains the supplement according to claim 1, wherein optionally a second container means contains a basal medium, wherein optionally a third container means contains embryonic stem cells.

52. A product of manufacture comprising one or more container means, wherein a first container means contains the medium according to claim 26 or 34, wherein optionally a second container means contains embryonic stem cells.

53. The product of manufacture according to any one of claims 49-52, wherein said product of manufacture is in a frozen state.

54. A method of expanding embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with the medium according to claim 26 or 34; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion said embryonic stem cells.

55. The method according to claim 54, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

5 56. A method of producing a transgenic animal, said method comprising

(a) cultivating embryonic stem cells in the medium according to claim 26 or 34;

10 (b) introducing a nucleic acid molecule into said embryonic stem cells;

(c) selecting a recombinant embryonic stem cell clone;

(d) expanding said recombinant embryonic stem cell clone to form a population;

15 (e) injecting an aliquot of said recombinant embryonic stem cell clonal population into a blastocyst;

(f) transferring said injected blastocyst into a host pseudopregnant female animal; and

(g) selecting transgenic offspring.

20 57. The method according to claim 56, wherein said cultivating further comprises

(a1) contacting said embryonic stem cells with the medium according to claim 26 or 34; and

25 (a2) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion said embryonic stem cells in serum-free culture.

58. The method according to claim 57 wherein said method comprises seeding said embryonic stem cells upon a layer of feeder cells.

59. A method of producing a transgenic animal, said method comprising

- 5 (a) cultivating embryonic stem cells in the medium according to claim 26 or 34;
- (b) introducing a nucleic acid molecule into said embryonic stem cells;
- 10 (c) selecting a recombinant embryonic stem cell clone;
- (d) expanding said recombinant embryonic stem cell clone to form a population;
- (e) co-culturing a small number of the embryonic stem cells with early stage embryos to form aggregates of embryos;
- 15 (f) transferring said aggregated embryos into a host pseudopregnant female animal; and
- (g) selecting transgenic offspring.

60. The method according to claim 59, wherein said cultivating further comprises

- 20 (a1) contacting said embryonic stem cells with the medium according to claim 26 or 34; and
- (a2) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion said embryonic stem cells in serum-free culture.

61. The method according to claim 60 wherein said method comprises seeding said embryonic stem cells upon a layer of feeder cells.

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62. A method of producing a recombinant protein from a transgenic animal, said method comprising

(a) cultivating embryonic stem cells in the medium according to claim 26 or 34;

(b) introducing a nucleic acid construct comprising a nucleic acid molecule which encodes a protein of interest encoding said protein into said embryonic stem cells;

(c) selecting a recombinant embryonic stem cell clone;

(d) expanding said recombinant embryonic stem cell clone to form a population of recombinant embryonic stem cells;

(e) injecting said recombinant embryonic stem cell clonal population into a blastocyst;

(f) transferring said injected blastocyst into a host pseudopregnant female animal;

(g) selecting transgenic offspring;

(h) raising said selected transgenic animal(s) under conditions suitable to promote the health of said transgenic animal; and

(i) isolating said recombinant protein from said transgenic animal.

63. The method according to claim 62, wherein said cultivating further comprises

(a1) contacting said embryonic stem cells with the medium according to claim 26 or 34; and

(a2) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells in serum-free culture.

64. The method according to claim 63, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

65. A method of producing a recombinant protein from a transgenic animal, said method comprising

(a) cultivating embryonic stem cells in the medium according to claim 26 or 34;

(b) introducing a nucleic acid construct comprising a nucleic acid molecule which encodes a protein of interest encoding said protein into said embryonic stem cells;

(c) selecting a recombinant embryonic stem cell clone;

(d) expanding said recombinant embryonic stem cell clone to form a population of recombinant embryonic stem cells;

(e) co-culturing a small number of the embryonic stem cells with early stage embryos to form aggregates of embryos;

(f) transferring said aggregated embryos into a host pseudopregnant female animal; and

(g) selecting transgenic offspring;

(h) raising said selected transgenic animal(s) under conditions suitable to promote the health of said transgenic animal; and

(i) isolating said recombinant protein from said transgenic animal.

66. The method according to claim 67, wherein said method further comprises

(a1) contacting said embryonic stem cells with the medium according to claim 26 or 34; and

(a2) cultivating said embryonic stem cells under serum-free conditions suitable to facilitate the expansion of said embryonic stem cells in serum-free culture.

67. The method according to claim 66, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

68. A method for controlling or preventing the differentiation of embryonic stem cells in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with the medium according to claim 26 or 34; and

(b) cultivating said embryonic stem cells under serum-free conditions suitable to control or prevent the differentiation of embryonic stem cells and facilitate the expansion of said embryonic stem cells in serum-free culture.

69. The method according to claim 68, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

70. The method according to claim 69, wherein said cultivating further comprises supplementing said medium with one or more factors which control or prevent the differentiation of said embryonic stem cells.

71. The method according to claim 70, wherein said factor is selected from the group consisting of leukemia inhibitory factor, steel factor, ciliary neurotrophic factor, and oncostatin M.

72. The method according to claim 71, wherein said factor is leukemia inhibitory factor.

73. The method according to claim 71, wherein said factor is steel factor.

74. The method according to claim 71, wherein said factor is ciliary neurotrophic factor.

75. The method according to claim 71, wherein said factor is oncostatin M.

76. A method of causing embryonic stem cells to differentiate into a particular type of cell in serum-free culture, said method comprising

(a) contacting said embryonic stem cells with the medium according to claim 26 or 34;

(b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture; and

(c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell.

77. The method according to claim 76, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

78. The method according to claim 76, wherein said cultivating said embryonic stem cells under conditions suitable to prevent the differentiation of and facilitate the expansion of said cells further comprises supplementing said culture medium with one or more growth factors which prevent differentiation of said embryonic stem cells.

79. The method according to claim 76, wherein said cultivating said expanded embryonic stem cells further comprises supplementing said culture medium with one or more growth factors which facilitate differentiation of said embryonic stem cells.

80. A method of providing differentiated embryonic stem cells, in serum-free culture, to a mammal, said method comprising

(a) contacting embryonic stem cells with the medium according to claim 26 or 34;

(b) cultivating said embryonic stem cells under conditions suitable to facilitate the expansion of embryonic stem cells in serum-free culture;

- (c) adding a differentiation factor or changing culturing conditions to induce differentiation of embryonic stem cells to form a different type of cell; and
- (d) introducing said differentiated cells into a mammal.

5 81. The method according to claim 80, wherein said method further comprises seeding said embryonic stem cells upon a layer of feeder cells.

82. The method according to claim 80, wherein said cultivating said embryonic stem cells under serum-free conditions suitable to prevent the differentiation of said cells further comprises supplementing said culture medium with one or more factors.

10 83. The method according to claim 82, wherein said factor is leukemia inhibitory factor.

15 84. The method according to claim 80, wherein said cultivating said expanded embryonic stem cells under serum-free conditions suitable to induce the differentiation of said cells further comprises supplementing said culture medium with one or more growth factors.

85. A method of obtaining embryonic stem cells in serum-free culture, said method comprising

- (a) isolating embryonic stem cells from blastocysts; and
- (b) cultivating said isolated embryonic stem cells in the medium according to claim 26 or 34.

20 86. A method of producing recombinant protein embryonic stem cells in serum-free culture, said method comprising

- (a) obtaining a recombinant embryonic stem cell containing a nucleic acid molecule which encodes a protein of interest;

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(b) culturing said embryonic stem cell in serum free culture to form a population of recombinant embryonic stem cells; and

(c) isolating said protein from said embryonic stem cells or from the medium in which said cells are cultured.

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87. The method according to claim 87, wherein said isolating further comprises

(c1) isolating said protein from said embryonic stem cells.

88. The method according to claim 86, wherein said isolating further comprises

(c1) isolating said protein from said harvested medium.

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e1

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